Relationships between serum serotonin, plasma cortisol and behavioral factors in a mixed-breed, -sex and -age group of pet dogs

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Abstract

Previously identified correlations between circulating cortisol and serotonin levels with aggressive, fearful or impulsive behavior in dogs have led to the suggestion that these measures be assessed as screening tools to aid in the identification of individual dogs with aggressive or fearful tendencies in applied settings, such as shelters or breeding programs. Previous studies investigating relationships between peripheral serotonin or cortisol and behavioral measures have several limitations, including single-breed sample groups, small sample sizes, and inconsistent methods used to collect behavioral data. This study used previously validated questionnaires to investigate relationships between owner-reported histories of dogs' behavior and peripheral cortisol and serotonin measures collected upon presentation to a novel environment, in a mixed-breed and mixed-age sample of dogs. No notable relationships were found between these measures in this group, indicating that circulating cortisol and serotonin are poor candidates for use in applied behavioral assessments for mixed breed pet dogs of varying ages. Differences between results reported here and previous literature are discussed.

Keywords: aggression; fear behaviors; cortisol; dog; impulsivity; serotonin

Introduction

Within the existing literature for canines, both plasma cortisol and serum serotonin have received attention for possible correlations with aggressive behavior traits in dogs. Three studies specifically investigated the correlation between low serum serotonin concentrations and owner-reported histories of aggression towards people or dogs (Rosado et al., 2010a; Leòn et al., 2012; Amat et al., 2013). While all of these studies concluded that aggressive dogs typically have lower serum serotonin concentrations than dogs with no history of problematic aggression, there was significant overlap of the mean serum concentrations of the aggressive (209.6–318.6 ng/ml) and nonaggressive sample groups (282.5–852.77 ng/ml) between studies. As such, existing data provide inconclusive evidence that serum serotonin measures are useful indicators of aggressive tendencies in an applied environment, and a reliable reference range would need to be determined before serum serotonin could be used to help identify pet dogs with aggressive tendencies.

Associations between plasma cortisol and behavioral traits are less straight forward than those for serum serotonin, as circulating cortisol is used as an indicator of acute stress in dogs. Even so, several associations have been identified between increased plasma cortisol concentrations and behavioral tendencies, including a history of aggression towards people (Rosado et al., 2010a), high scores on 'emotionality' and fear-related behavioral measures (Foyer et al., 2016), general behavior problems in puppies adopted from shelters, as reported by owners six months post adoption (Hennessy et al., 2001), and fearful behaviors in dogs newly admitted to shelters, during interactions with unfamiliar humans (Shiverdecker et al., 2013). Together, these studies indicate a possible association between increased plasma cortisol concentration when sampled in novel or stressful environments, and fearful or

aggressive behavioural tendencies in domestic dogs.

For peripheral measures of cortisol and serotonin to be used in conjunction with behavioral measures to assess behavior of pet dogs in an applied environment, it first needs to be demonstrated that previously identified associations between problematic behaviors and these measures exist in a sample of dogs of mixed breeds and varying ages, as would be the case in a typical pet population. Additionally, reference ranges for both circulating cortisol and serotonin, and sampling guidelines must be developed prior to applied use of these tools for behavioral assessments. Although alternative measures of circulating cortisol and serotonin such as urine or saliva analysis are more feasible in most applied environments, these measures are influenced by a larger number of external factors than direct blood sampling (Cobb et al., 2016; Kema et al., 1992; Marc et al., 2011). As such, direct measurement of blood is ideal for the purpose of establishing a relationship between behavioral tendencies and peripheral concentrations of cortisol or serotonin. This study examined relationships between owner-reported behavioral histories and serum serotonin and plasma cortisol concentrations, in a mixed-breed and mixed-age sample of pet dogs presented by their owners to a novel environment.

Material and methods

Recruitment

This study was part of a larger research project investigating applied personality assessments in pet dogs. One hundred and seven dog owners were recruited through internet forums, social media sites and hard-copy posters displayed around the La Trobe University campus in

Bundoora, Victoria, Australia. Participation was voluntary, with interested parties asked to contact the researcher via e-mail. Interested owners of healthy dogs, who were 18 years of age or over, were fluent in English and had owned their dog for at least six months, could participate. Owners were asked to fast their dogs for at least 6 h prior to their appointment time, while allowing free access to water. Samples were taken at one of three testing facilities, one in suburban Melbourne and two in regional Victoria, Australia.

Blood sample collection

Dogs were transported to the testing facility by their owners. Upon arrival, the procedure was explained to owners, and dogs were allowed to investigate the immediate area. Dogs were then restrained either by their owners or by the researchers in the presence of the owners. All samples were collected within 3 min of the onset of restraint to minimize the effects of restraint stress, in accordance with the method described by Coover et al. (1979). Blood samples were collected from the cephalic vein using the VACUETTE blood collection system, with 22G needles and 2-ml pediatric collection tubes (Greiner Bio-One, Kremsmünster, Austria; part ref. 454024 K2EDTA for plasma, and 454096 Z Serum Clot Activator for serum). Samples were kept at room temperature (21–23°C) for a maximum of 90 min prior to being spun at $2000 \times g$ for 10 min. The supernatant was aliquoted into fresh tubes and stored at -20°C for up to 6 months prior to analysis.

Owner-report questionnaires

Owners were asked to fill out a questionnaire describing the lifestyle and typical behavior of their dogs. The questionnaire consisted of three sections, including two previously published questionnaires:

1. Custom-written questions relating to dog and owner demographics such as age, sex and training history of dog, and education and dog-related work experience of owners (see Supplementary Materials S1);
2. Dog Impulsivity Assessment Scale (DIAS; Wright et al., 2011), which includes measures of self-control, neophobia and response to novelty, impulsive aggression and responsiveness to humans;
3. Canine Behavioural Assessment and Research Questionnaire (CBARQ; Hsu and Serpell, 2003), which features 13 factors designed to assess behavioral responses to a broad range of contexts commonly encountered by pet dogs.

Cortisol and serotonin measurement

Due to sample volume restricting analysis for both cortisol and serotonin in a subset of dogs, only 80 of the 107 dogs were used for serum serotonin measurements. Samples were analyzed in duplicate for cortisol and serotonin using commercial ELISA kits, in accordance with the manufacturer's instructions (Enzo Life Sciences, Farmingdale, NY, USA; Cortisol ELISA kit ADI-900-071; ENZO Serotonin ELISA kit ADI-900-175). Plasma samples were diluted 1:10 prior to analysis for cortisol, and the supplied Steroid Displacement Reagent was used with all samples. Serum samples were diluted 1:17 prior to analysis for serotonin concentrations. Standard curves for both serotonin and cortisol assays were determined using an automated 4-parameter-logistic curve-fitting program in Microsoft Excel (available from www.rheumatologie-neuss.net/index_files/ELISA%20AUTO%20CURVE%20FIT.xls). All

standard curve regressions used for calculation of sample concentrations were a minimum of $r^2=0.99$. Intra-assay coefficients of variation were 2.9–7.7%, and inter-assay coefficients of variation were 5.4–11.2%.

Statistical analysis

Statistical analysis was carried out using IBM SPSS version 22.0. Calculated factor scores for DIAS and CBARQ were determined according to published guidelines (Hsu and Serpell, 2003; Wright et al., 2011). Correlations between plasma cortisol concentrations, serum serotonin concentrations and questionnaire factors were analyzed using Spearman's rank order correlations. Kruskal-Wallis tests were used to compare mean plasma cortisol and serum serotonin concentrations for groups split according to questionnaire factors relating to aggression, fear and impulsivity; CBARQ factor scores were categorized as low (0–1), medium (1.1–2.9), and high (>3). DIAS factor scores were categorized as low (0–0.3), medium (0.31–0.6) and high (>0.6), and also according to the sample distribution with low (<33rd percentile), medium (33rd to 67th percentile) and high (>67th percentile) categorisations.

Results

Participants

Of the 107 owners that participated in the study, 100 were female and all identified themselves as either the sole or shared primary carer of the dog presented. Owners were between 18 and 70 years of age and 87.5% of participants had formal education beyond

secondary college. More than half (55%) of the owners reported formal work or volunteer experience with dogs, coming from a variety of backgrounds, including the veterinary, dog training, and welfare sectors, and from pet service providers such as pet shops or groomers. Many participants had work experience in more than one area. Participant households typically consisted of single adults or couples (75%) and no children under 18 years of age (76%).

Dogs

Sample dogs were aged between 8 months and 11 years, with an average age of 4 years. The sample contained more purebreds (56%) than mixed-breed dogs (44%), with 25 different pure breeds of dog represented in the sample. The sample contained 56 male dogs (90% of which were desexed) and 51 female dogs (80% of which were desexed). Dogs were acquired primarily as puppies (71%) to fulfill the role of pets or companions (91%), although many owners also reported showing, breeding, dog sports and working or service roles as secondary reasons for acquiring the dog. Roles of dogs primarily acquired for purposes other than companionship included showing (three dogs), dog sports (two dogs) and search and rescue work (one dog). Dogs were typically sourced from breeders with (28%) or without (16%) pedigree papers, or from welfare sources (30%). Other sources of dogs included pet shops (9%), 'free to good home' advertisements (6%), family or friend (5%), 'bred myself' (2.5%), or 'other' (3.5%). Most dogs either lived in a house with a backyard (73%), or on acreage including working farms (24%).

Cortisol and serotonin

Using Spearman's rank order correlation analysis, no correlations were found between behavior questionnaire factors from owner reports and circulating serotonin ($N=80$) and cortisol concentrations ($N=107$) in this sample of pet dogs (Table 1). Both CBARQ 'Separation-related Problems' and CBARQ 'Touch Sensitivity' showed a slight positive correlation with plasma cortisol that approached statistical significance in this sample group. Kruskal-Wallis analysis of variance of sample groups of pet dogs with low, medium and high factor scores for fear-, aggression- and impulsivity-related behavior traits, showed no significant differences in serum serotonin ($N=80$) or plasma cortisol concentrations ($N=107$) between these groups (Figures 1 and 2, and Supplementary Materials S2).

Discussion

Using a mixed-aged, mixed-breed sample of pet dogs from the community, no significant correlations were found between aggressive, fearful or impulsive behavioral traits and circulating levels of cortisol or serotonin when presented to a novel environment. A weak positive correlation that approached statistical significance was observed between plasma cortisol concentration and the CBARQ factors 'Touch Sensitivity' and 'Separation-related Problems'. In addition, no differences were observed in mean serum serotonin and plasma cortisol concentrations of dogs with varying histories of aggressive, fearful or impulsive behavior, as reported by their owners. These results contradict earlier findings that circulating cortisol and serotonin levels in pet dogs presented to a novel environment reflect incidence of aggressive and fearful behaviors in domestic dogs.

Given that blood sample collection and analysis methodology were similar to those used in previous studies, and observed concentrations of serotonin and cortisol fell within normal

ranges for healthy dogs, there are three likely explanations for the lack of difference between groups found in this study: (1) variation in peripheral serum and cortisol in dogs associated with fearful or aggressive tendencies may be masked by other sources of variation in these measures, or exposure to novelty and unfamiliar people is not stressful enough to elicit a response in aggressive or fearful dogs; (2) behavioral measures used by researchers may lack the consistency or resolution required to identify fearful or aggressive tendencies; or (3) there may simply be no meaningful relationship between peripheral serotonin and behavioral tendencies in domestic dogs at a population level.

Other forms of variation affecting circulating serotonin and cortisol levels mask differences related to behavioral traits

Serotonin. Despite methods for serum collection and serotonin analysis being comparable across studies, mean values reported in existing literature and in this study vary widely with a substantial degree of overlap between sample groups based on behavioral histories. One possible confounding source of variation for this relationship is breed. Van den Berg et al. (2005) identified breed-related variation in the frequency of haplotypes for the serotonin receptor 1A gene. Given that links between central serotonergic system function and aggressive and impulsive behavior in mammals are widely accepted (Montoya et al., 2012; Seo et al., 2008), and variation in these behavioral tendencies exists between domestic dog breeds (Duffy et al., 2008; Fadel et al., 2016) and breed groups (Turcsan et al., 2011), it is likely that some variation in these factors exists when different breeds are considered.

In addition to breed-related variation, environmental input and individual learning have been shown to have a substantial impact on aggressive (Blackwell et al., 2008), impulsive (Lit et

al., 2010) and fearful (Howell et al., 2015) behavior in dogs. Furthermore, environmental factors such as exposure to toxins (Aldridge et al., 2005) and enriched versus impoverished living conditions (Chourbaji et al., 2012) have been linked with changes in the serotonergic system in mammals. Given the variety of environments that pet dogs originate from, it is likely that at least some of these factors will affect either behavior or serum serotonin levels as much or more than any inherent co-variation that may exist between them.

Cortisol. While the values reported here fall on the low boundary of normal plasma cortisol range for domestic dogs as described by Johnston and Mather (1978), they are notably lower than is typically reported for domestic dogs in recent literature. Both Rosado et al. (2010a) and Hennessy et al. (2001) report sampling in situations that pet dogs may find stressful—during presentation to a veterinary clinic for examination (Rosado et al., 2010a), or while housed in a welfare shelter (Hennessy et al., 2001). Dogs in the current study were presented to a quiet novel environment by their owners, and samples were taken by an unfamiliar person within 3 min of the onset of restraint while in the presence of their owners. Additionally, while some dogs in this study were reported by their owners to have performed aggressive or fearful behavior towards strangers (up to and including barking, lunging and attempting to bite), none were categorized as having high levels of aggression or fearful behavior towards strangers using the CBARQ factors ‘Stranger-directed Fear’ and ‘Stranger-directed Aggression’. Given that pet dogs demonstrate secure-base attachment towards their owners (Palmer and Custance, 2008), it is possible that dogs in this study were simply less acutely stressed in this environment than those in the two previous studies, resulting in lower plasma cortisol values overall. Similarly, aggressive or fearful dogs may have found the sampling environment of previous studies more stressful than sociable ones, and so experienced a higher level of acute stress in addition to that associated with restraint. This

effect may also explain the weak positive correlation observed here between plasma cortisol and the CBARQ factors ‘Touch Sensitivity’ and ‘Separation-related Problems’, although the lack of correlation between plasma cortisol concentrations and the CBARQ factors ‘Stranger-directed Fear’ and ‘Nonsocial Fear’ is unexpected and contradictory to this explanation, given the context in which sampling occurred. Acute stress associated with the environment and sampling method is a possible cause for some of the variation reported between aggressive and nonaggressive groups of dogs in previous studies.

It is also possible that differences in baseline levels of plasma cortisol exist between aggressive and sociable dogs, but the effect of acute stress masked any differences in baseline cortisol present between the sample groups. Our current understanding of the cortisol–aggression relationship in mammals is that cortisol is a modulator for androgen-related aggression (reviewed by Montoya et al., 2012); that is, individuals with high testosterone and low cortisol are consistently more likely to display both impulsive and instrumental forms of social aggression than those with high cortisol. This relationship is the opposite of what has been described in the canine literature, and is unlikely to explain trends seen in the pet dog population given that most pet dogs in Australia and the USA are surgically sterilized (Animal Medicines Australia Pty Ltd., 2016; Clancy and Rowan, 2003).

Use of published questionnaires for collecting information about the behavioral history of dogs

While previous authors have described the use of recognized methods to collect and interpret behavioral data, these are not replicable due to lack of information provided in these publications. The definition of aggression, the frequency of aggressive incidents, and the

target of aggression varied between studies and was sometimes not reported at all (Çakiroğlu et al., 2007; Leòn et al., 2012; Rosado et al., 2010a; Amat et al., 2013). We collected behavioral information and defined problematic behavior traits using published, validated questionnaires for general dog behavior (CBARQ; Hsu and Serpell, 2003) and impulsivity (DIAS; Wright et al., 2011). Even so, formal experience or training in behavior and method of data collection have both been shown to affect the validity and reliability of behavioral information collected about animals (Diesel et al., 2008; Highfill et al., 2010; Rayment et al., 2016), particularly in relation to aggressive behavior in dogs (Tami and Gallagher, 2009). It is therefore probable that these methodological differences are responsible for some of the observed variation between studies. In future, closer attention needs to be paid to the methods used to collect information about the behavioral tendencies of pet dogs, as this has been repeatedly identified as a significant source of variation between studies.

Circulating serotonin is not tightly correlated with central activity of the serotonergic system

The lack of consistency in studies investigating associations between circulating serotonin and canine behavior traits may simply indicate that there is not a noteworthy relationship between these measures in dogs. Increased serotonergic function in the brain is thought to have an inhibitory effect on aggression in mammals (Coccaro et al., 2015). However, this relationship is complex and still not fully understood, with the type and context of aggression, individual genetics, and varying responses to drug treatment all affecting this relationship (Carrillo et al., 2009). Until recently, most work investigating links between serotonin and aggression has come from the fields of medicine and psychology; this work first described and then attempted to explain the effects of selective serotonin uptake inhibitors (SSRIs) and other medications that increase circulating serotonin (and therefore central serotonergic

function) and decrease anxious and aggressive behavior (Walsh and Dinan, 2001). The veterinary medicine field has followed suit with studies describing the effects of SSRIs on aggression (Rosado et al., 2011), compulsive behavior (Irimajiri et al., 2009), and separation anxiety and phobias in dogs (Ibáñez and Anzola, 2009; Karagiannis et al., 2015; Sherman and Mills, 2008). As our understanding of the complexity of the serotonergic system deepens, the focus of research into links with behavioral measures has moved towards foundation and preclinical studies. These studies investigate the function of serotonin receptors (Olivier and van Oorschot, 2005; Popova and Naumenko, 2013; Popova et al., 2007) and transporters (Rosado et al., 2010b; Talati et al., 2017; Vermeire et al., 2012), and genetic variation that may underlie individual differences in these functions (Landrø et al., 2015; Plieger et al., 2017; Stoltenberg et al., 2012; Våge et al., 2010). It is now understood that while serum serotonin levels may be useful to monitor a patient's response to drug therapy, both peripheral serotonin levels and serotonin metabolite levels in the cerebrospinal fluid do not closely mirror central serotonergic system function or correlate with behavioral trends at an individual level (Duke et al., 2013; Taciak et al., 2018). In addition, the exact process by which SSRIs and other drugs that increase circulating serotonin levels affect individual behavior is still not fully understood (Duke et al., 2013; Taciak et al., 2018). Given the complexity of the relationship between behavior and serotonergic system function, it is unlikely that feasible and reliable measures of serotonergic function that relate to behavioral characteristics will become available soon for use as selection aids for behavior tendencies in an applied setting.

Conclusion

We examined relationships between circulating cortisol and serotonin with behavioral histories of aggression, fearfulness and impulsivity in a mixed-age, mixed-sex, and mixed-breed group of pet dogs. Samples were collected in a novel environment while in the presence of the owner. No correlations were found between serotonin, cortisol and behavioral measures including those related to fear and aggression towards strangers or fear of novel environments, although weak correlations approaching statistical significance were identified between circulating cortisol and the two CBARQ factors ‘Separation-related Problems’ and ‘Touch Sensitivity’. No notable differences in cortisol and serotonin measures were observed between dogs grouped by owner reports of fearful, impulsive, or aggressive behavior, and variation of cortisol and serotonin values within behavior groups was high. When examined in the context of existing literature describing relationships between cortisol and serotonin with behavior in mammals, this indicates that circulating cortisol and serotonin measures are unlikely to aid in the identification of pet dogs with fearful and aggressive tendencies in applied settings. Lack of consistency between studies in the methods used to categorize behavioral tendencies in dogs hinders useful comparisons of results. Furthermore, it is possible that previous studies reporting a relationship between circulating cortisol and reported histories of aggression in pet dogs may be explained through stress caused by the sampling environment.

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Ethics statement

This study is compliant with the Australian National Statement on Ethical Conduct in Human Research, and the *Australian code of practice for the care and use of animals for scientific purposes* 7th edition (2004). This study was approved by the La Trobe University Animal Ethics Committee (approval number AEC12-26) and the La Trobe University Human Ethics Committee (approval number FHEC12/R42).

Conflict of interest statement

The authors have nothing to disclose.

Authorship statement

All authors have approved the final article. The idea for the paper was conceived by Diana Rayment and Linda Marston. The experiments were designed by Diana Rayment, Richard Peters, Linda Marston and Bert De Groef. The experiments were performed by Diana Rayment. The data were analyzed by Diana Rayment, Richard Peters and Bert De Groef. The paper was written by Diana Rayment, and edited by Richard Peters, Linda Marston and Bert De Groef.

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Table 1. Spearman's rank order correlations between Canine Behavioural Assessment and Research Questionnaire (CBARQ) factors, Dog Impulsivity Assessment Scale (DIAS) factors, plasma cortisol concentrations ($N=107$), and serum serotonin concentrations ($N=80$) in a sample of healthy dogs of mixed breeds, sex and age. Values listed are rho values, followed by P values between brackets.

	Plasma cortisol (ng/ml)	Serum serotonin (ng/ml)
CBARQ Stranger-directed aggression	-0.10 (0.32)	-0.1 (0.38)
CBARQ Dog-directed aggression	-0.06 (0.51)	-0.03 (0.79)
CBARQ Stranger-directed fear	-0.13 (0.20)	-0.02 (0.88)
CBARQ Dog-directed fear	0.00 (0.96)	0.07 (0.51)
CBARQ Non-social fear	-0.02 (0.84)	-0.02 (0.86)
CBARQ Separation-related problems	0.18 (0.06)	-0.19 (0.09)
CBARQ Touch sensitivity	0.18 (0.06)	-0.19 (0.09)
DIAS Behavioural regulation	0.00 (0.99)	-0.14 (0.21)
DIAS Response to novelty	0.03 (0.78)	-0.17 (0.14)

Figure 1. Kruskal Wallis one-way analysis of variance for (A) mean plasma cortisol ($N=107$) and (B) mean serum serotonin ($N=80$) concentrations for pet dogs separated by Canine Behaviour Assessment and Research Questionnaire (CBARQ) and Dog Impulsivity Assessment Scale (DIAS) factors. Values indicate means $\pm$ standard error. Numbers within bars indicate sample size. CBARQ factor scores were categorized as low (0–1), medium (1.1–2.9), or high (>3), and DIAS factor scores were categorized as low (0–0.3), medium (0.31–0.6) or high (>0.6). No significant differences were found between any groups (see Supplementary Materials S2).

Figure 2. Kruskal-Wallis one-way analysis of variance for **(A)** mean plasma cortisol ($N=107$) and **(B)** mean serum serotonin ($N=80$) concentrations for pet dogs separated by Dog Impulsivity Assessment Scale (DIAS) factors. Values indicate means $\pm$ standard error. Numbers within bars indicate sample size. DIAS factor scores were categorized according to distribution of factor scores, as low (<33rd percentile), medium (33rd to 67th percentile) or high (>67th percentile). No significant differences were found between any groups (see Supplementary Materials S2).

A **B**

Supplementary material: Custom-written questions completed by participating owners, relating to dog and owner demographics such as age, sex and training history of dog, and education and dog-related work experience of owners.

Section 1a) General Information

1. Indicate your approximate age (please circle)

18 to 25 years	26 to 40 years	41 to 55 years	56 to 70 years	70 years plus
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2 Indicate your gender. (please circle) Male / Female

3 What type of accommodation do you live in? (please circle)

House with yard	House with small/no yard	Apartment/Unit
Townhouse	House with acreage	Working farm
Other (please describe) _____		

4 What is your postcode? _____

5 Please indicate how many people in each category live with the dog.

Males (65 years plus)_____	Females (65 years plus)_____
Males (18 to 65 years)_____	Females (18 to 65 years)_____
Males (11 to 17 years)_____	Females (11 to 17 years)_____
Males (5 to 10 years)_____	Females (5 to 10 years)_____
Males (0 to 4 years)_____	Females (0 to 4 years)_____

6 What is the relationship of these people to you?(circle more than one if appropriate)

Unrelated	Parent	Partner/Spouse	Children	Other family member
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7 Do you regularly (at least once a week) have a child/children visiting the house that are in the following age groups? (please circle applicable ages)

0-2 years	3-5 years	6-10 years	11-15 years
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8 What is your highest level of education? (please circle)

Secondary	TAFE qualification	Trade qualification
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Tertiary (degree)	Post graduate	Other _____
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9 Please indicate your approximate annual household income (please circle)

Less than \$50,000	\$50,000 to \$100,000	\$100,000 to \$150,000	\$150,000 plus
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Section 1b) General information specific to your dog.

10 How many dogs have you owned before? (please circle)

None	1-2	3-10	>10
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11 Do you own any other animals? (please circle) Yes / No

If so, please specify how many of each type

Dog _____	Cat _____
Rodent _____	Bird _____
Horse/Pony _____	Livestock _____
Reptile _____	Fish _____
Other (please specify) _____	

12 Are you the primary carer of this dog? (please circle) Yes / No / Shared

13 How long have you owned this dog? _____ years _____ months

14 How old was this dog when you got it? _____ years _____ months

15 Where did you obtain this dog from? (please circle)

Breeder (with ANKC papers)	Breeder (no ANKC papers)	Pet Shop
'Free to good home'	Family/Friend	Shelter/Rescue
Found it	Other (please specify) _____	

16. What was the primary purpose for which this dog was obtained (please circle)?

Family pet	Companionship	Security	Breeding
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Showing	Dog sports	Other (specify) _____
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17. Is your dog a mixed breed or pure bred? (please circle) Mixed / Purebred

18. What breed is your dog (if mixed, what breed does your dog most resemble)?

19. What sex is your dog? (please circle)

Male (entire)	Male (desexed)	Female (entire)	Female (desexed)
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20. On an average weekday, how long is you dog left alone (please circle)?

0-2 hours	3-6 hours	6-12 hours	More than 12 hours
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21. On an average weekend, how long is your dog left alone (please circle)?

0-2 hours	3-6 hours	6-12 hours	More than 12 hours
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22. Please describe how you normally exercise your dog?

23. During an average week, how long would you spend exercising your dog?
_____ hours

24. During an average week, how long do you spend relaxing with your dog?
_____ hours

25. Did you take your dog to puppy classes? (please circle) Yes / No

26. Have you attended any formal dog training lessons with your dog? Yes / No

27. If you have attended dog training, which type of school did you attend? (please circle)

Private business	Breed club	Community-based club
Other (please specify) _____		

28. Do you compete with your dog? Yes / No

If so, in which activities do you compete? (please circle)

Conformation shows	Breed-specific skills/games	Obedience
Flyball	Lure coursing/racing	Hunting

Herding	Agility	Freestyle obedience (dancing)
Field trials	Schutzhund	Tracking
Other (please specify) _____		

29 During an average week, how long do you spend training your dog? _____hrs

30 Which of the following activities do you do with your dog (please circle)?

Swimming	Take to shops/café's	Take to beach
Camping	Hiking	Hunting
Go to dog park	Biking	Sleep in bed together
Play on playground equipment	Take for car rides	Take to work
Play tug	Play chase	Play fetch
Take visiting to see friends	Herding	Dress up
Therapy dog work (please specify) _____		
Other activities (please specify) _____		

31 Is your dog micro-chipped? (please circle) Yes / No

32 Is your dog up to date with its vaccinations? (please circle) Yes / No

33 Describe the behaviours of your dog that you like the most.

34 Which of your dog's behaviours would you change if you could? (please describe)

35 Please describe in your own words how your dog reacts in the following situations.

- a. When meeting new dogs
- b. When people unknown to the dog visit your house
- c. When something unexpectedly gives your dog a fright

Supplementary Table. Statistical values for Kruskal-Wallis one-way analysis of variance for **(A)** mean plasma cortisol (ng/ml) ($N=107$) and **(B)** mean serum serotonin (ng/ml) ($N=80$) for pet dogs separated by Canine Behaviour Assessment and Research Questionnaire (CBARQ) and Dog Impulsivity Assessment Scale (DIAS) factors.

A

	χ^2	Degrees of freedom	Asymptotic significance
CBARQ Stranger-directed aggression	1.592	1	0.207
CBARQ Dog-directed aggression	0.362	1	0.547
CBARQ Stranger-directed fear	0.689	1	0.407
CBARQ Dog-directed fear	0.370	2	0.831
CBARQ Separation-related problems	3.394	1	0.065
CBARQ Non-social fear	0.530	1	0.467
DIAS Behavioral regulation	0.940	2	0.625
DIAS Response to novelty	1.518	2	0.468

B

	χ^2	Degrees of freedom	Asymptotic significance
CBARQ Stranger-directed aggression	0.277	1	0.598
CBARQ Dog-directed aggression	0.000	1	1.000
CBARQ Stranger-directed fear	0.100	1	0.752
CBARQ Dog-directed fear	0.716	2	0.699
CBARQ Separation-related problems	3.217	1	0.073
CBARQ Non-social fear	0.547	1	0.459
DIAS Behavioral regulation (score)	2.904	2	0.234
DIAS Response to novelty (score)	2.457	2	0.293
DIAS Behavioral regulation (percentile)	0.417	2	0.813
DIAS Response to novelty (percentile)	2.152	2	0.341